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Loss of phospholipase PLAAT3 causes a mixed lipodystrophic and neurological syndrome due to impaired PPAR γ signaling

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69 **Abstract**

70 PLAAT3 is a phospholipid-modifying enzyme predominantly expressed in neural and white
71 adipose tissue (WAT). It is a potential drug target for metabolic syndrome, as *Plaat3* deficiency
72 in mice protects against diet-induced obesity. We identified seven patients from four unrelated
73 consanguineous families, with homozygous loss-of-function variants in *PLAAT3*, who
74 presented with a lipodystrophy syndrome with loss of fat varying from partial to generalized
75 and associated with metabolic complications, as well as variable neurological features including
76 demyelinating neuropathy and intellectual disability. Multi-omics analysis of mouse *Plaat3*^{-/-}
77 and patient-derived WAT showed enrichment of arachidonic acid-containing membrane
78 phospholipids and a strong decrease in the signaling of PPAR γ , the master regulator of
79 adipocyte differentiation. Accordingly, CRISPR/Cas9-mediated *PLAAT3* inactivation in human
80 adipose stem cells induced insulin resistance, altered adipocyte differentiation with decreased
81 lipid droplet formation and reduced the expression of adipogenic and mature adipocyte markers,
82 including PPAR γ . These findings establish PLAAT3 deficiency as a hereditary lipodystrophy
83 syndrome with neurological manifestations, caused by a PPAR γ -dependent defect in WAT
84 differentiation and function.

85 **MAIN**

86 Human PLAAT3, previously known as phospholipase A2, group XVI (PLA2G16), is part of a
87 family of acyltransferases and phospholipases, which catalyze phospholipase A (PLA) and
88 acyltransferase (AT) activities.⁽¹⁾ In mice, *Plaat3* is highly expressed in white adipose tissue
89 (WAT) where it exhibits PLA₁ and PLA₂ activities, which hydrolyze fatty acids linked to the
90 *sn-1* or *sn-2* positions of membrane phospholipids.⁽¹⁻⁴⁾ *Plaat3*-deficient mice are resistant to
91 diet-induced obesity, and PLAAT3 was therefore suggested as a potential therapeutic target for
92 metabolic syndrome.⁽⁵⁾

93 Lipodystrophy syndrome is characterized by generalized or partial lipoatrophy with
94 reduced WAT due to a defect in adipose tissue storage of triglycerides (TG).⁽⁶⁾ This results in
95 lipid infiltration of non-adipose tissues leading to insulin resistance, increased liver glucose
96 production, hypertriglyceridemia, and hepatosteatosis. Approximately 30 genes have been
97 implicated in hereditary lipodystrophy syndrome.^(7, 8) Although these disorders often remain
98 genetically unexplained, there is growing interest in identifying their molecular bases to
99 improve genetic counseling, personalize treatment,^(9, 10) and uncover signaling pathways
100 involved in diabetes, hepatosteatosis, or metabolic syndrome.^(9, 10) Patients with lipodystrophy
101 syndrome show striking metabolic similarities with obese subjects who display an incapacity
102 to store surplus energy in overwhelmed WAT resulting in ectopic lipid accumulation.

103 In this study, we combined homozygosity mapping, whole-exome and whole-genome
104 sequencing (WES/WGS) to identify homozygous null variants in *PLAAT3* as a cause of
105 lipodystrophy syndrome with neurological features. We further assessed the metabolic
106 consequences of the loss of PLAAT3 using a multi-omics approach in murine and human WAT.
107 The impact of the loss of PLAAT3 activity on human adipocyte differentiation and function
108 was also evaluated using a CRISPR-Cas9-mediated genome-editing approach.

109

110 **RESULTS**

111 **Identification of homozygous *PLAAT3* null variants in seven patients**

112 In a consanguineous family with three children (patients 1-3; family 1) (Figure 1a) presenting
113 with a lipodystrophy syndrome combining metabolic and neurological manifestations, we
114 performed SNP array-based homozygosity mapping in the three affected and two unaffected
115 siblings. The largest homozygous region (~43 Mb), exclusively shared between the affected
116 siblings, was found on chromosome 11p11.2-q14.2 and contained the *BSCL2* gene, implicated

117 in congenital generalized lipodystrophy type 2 (CGL2) (Supplementary Table 1). Normal
118 Sanger sequencing of *BSCL2* coding exons, followed by normal cDNA analysis of peripheral
119 leukocytes, excluded *BSCL2* as the causal gene. WES was performed in the three affected
120 sisters and two unaffected siblings, but we found no homozygous pathogenic single nucleotide
121 variants in the region of interest (Supplementary Table 2). Copy number variant analysis of
122 WES data using ExomeDepth⁽¹¹⁾ revealed a homozygous 103 bp deletion corresponding to exon
123 2 of the *PLAAT3* gene (NM_001128203.2) exclusively in the three affected sisters (Figure 1b
124 (upper panel), Supplementary Table 3). The deletion was located within the ~43 Mb shared
125 homozygous region and was confirmed by low-pass whole genome sequencing (CNV-seq).⁽¹²⁾
126 WGS in one unaffected and two affected sisters determined the genomic deletion breakpoints
127 (chr11: 63597894-63602986) revealing a homozygous 5092 bp deletion (Figure 1b (lower
128 panel)). Deletion of *PLAAT3* exon 2 (c.16-4823_118+167del) results in a frameshift leading to
129 a premature termination codon (p.(Pro6ValfsTer15)) (Figure 1c). RNA sequencing confirmed
130 that the molecular defect induced nonsense-mediated decay (NMD) (Supplementary Figure 1).

131 Using the GeneMatcher platform,⁽¹³⁾ we identified a female patient (patient 4; family 2)
132 (Figure 1a) in whom WES revealed a homozygous single base duplication in exon 3 of *PLAAT3*
133 leading to a frameshift and premature termination codon, predicted to induce NMD
134 (c.286dupG, p.(Ala96GlyfsTer16)) (Figures 1b, c).⁽¹³⁾ Through data sharing we found three
135 additional patients from two unrelated families (patient 5; family 3, patients 6-7; family 4)
136 (Figure 1a) in which WES identified a homozygous nonsense variant (c.339C>A;
137 p.(Cys113Ter)) in exon 3 of *PLAAT3* (Figure 1c). The variants identified in families 2, 3 and 4
138 were confirmed by Sanger sequencing (Figure 1b). Only the p.(Cys113Ter) variant was present
139 in gnomAD v3.1.2 (in one individual of African origin in the heterozygous state).

140

141 **Loss of *PLAAT3* is associated with a lipodystrophy syndrome**

142 *Family 1.* Three sisters (patients 1-3), born from consanguineous parents of Turkish origin,
143 presented with lipoatrophy of limbs and trunk and lipohypertrophy in the submental and
144 posterior cervical region in patients 2 and 3 (Figure 1a). The patients were lean with a body-
145 mass index (BMI) ranging from 19.6 to 20.5 kg/m². In patient 1 the lipoatrophy was confirmed
146 by dual X-ray absorptiometry with a total fat mass of 13.8% (Figure 2a). Serum leptin levels in
147 patient 1 and patient 2 were decreased (Supplementary Table 4). Other clinical signs typical for
148 lipodystrophy syndrome included muscle hypertrophy, insulin-resistant diabetes with
149 hyperinsulinemia, *acanthosis nigricans* and increased fasting glucose, hypertriglyceridemia
150 with low HDL-cholesterol, liver steatosis, and polycystic ovary syndrome with hirsutism

151 (Figure 2b, Table 1, Supplementary Table 4). Neurological symptoms comprised migraine
152 (patient 1), demyelinating neuropathy (patient 1) and intellectual disability (patient 2) (Table 1,
153 Supplementary Table 4). Patient 1 presented with unilateral carpal tunnel syndrome. Additional
154 features included generalized musculoskeletal pain, hypertension, subclinical hypothyroidism,
155 precocious puberty and acromegalic features (Supplementary Table 4). Short stature was seen
156 in all three affected siblings, which was probably constitutional given the short stature in both
157 unaffected parents (mother: 158 cm, father: 165 cm).

158 *Family 2.* Patient 4 (female) was born from Algerian first-degree cousins (Figure 1a). She
159 presented with an android habitus with generalized muscle hypertrophy and lipoatrophy in the
160 limbs with a relative accumulation of adipose tissue in the face, neck and submental region
161 resulting in a cushingoid appearance (Figure 2c). She had a BMI of 24.6 kg/m² with insulin-
162 resistant diabetes, hypertriglyceridemia with low HDL-cholesterol, liver steatosis, polycystic
163 ovary syndrome and hirsutism. She complained of chronic muscle pain and was diagnosed with
164 demyelinating neuropathy (Table 1). Arterial hypertension, transient hypothyroidism, carpal
165 tunnel syndrome, obstructive sleep apnea syndrome and glomerulopathy without renal
166 insufficiency were observed (Supplementary Table 4). Recently, she was diagnosed with
167 thyroid vesicular carcinoma requiring thyroidectomy.

168 *Family 3.* Patient 5 (male) was born from Algerian first-degree cousins (Figure 1a). His mother
169 had two early miscarriages before his birth. He presented with generalized lipodystrophy,
170 muscular hypertrophy, insulin-resistant diabetes, and liver steatosis (Figure 2d, Table 1). The
171 patient was lean with a BMI of 20.7 kg/m² and a body fat percentage of 10.1% (Table 1,
172 Supplementary Table 4). Serum leptin levels were very low. He developed gynecomastia at the
173 age of 12 years, which persisted after puberty (Figures 2d, e). Sex hormone levels were normal,
174 with slightly elevated free testosterone levels due to decreased sex hormone binding globulin
175 (SHBG) levels associated with insulin resistance. LH was within the normal range, FSH was
176 slightly upregulated with low inhibin B levels, suggesting spermatogenic insufficiency
177 (Supplementary Table 4). He presented with facial dysmorphism including low-set and
178 posteriorly rotated ears, prominent nose and forehead, high nasal bridge and prognathism.
179 Musculoskeletal manifestations included thoracic hyperkyphosis, *genu valgum*, *talipes*
180 *equinovarus*, retraction of Achilles tendons, hammer toes and bilateral syndactyly of the second
181 and third toes (Figures 2d-g). The patient presented with a rather short stature which was also
182 seen in his unaffected parents (Supplementary Table 4). During childhood, advanced bone age
183 with bone hypermineralization (Z-score >3.5 SD) was observed. Neurological features

184 consisted of psychomotor delay, intellectual disability, behavioral problems, demyelinating
185 neuropathy and spastic gait (Table 1). MRI of the brain was normal.

186 *Family 4.* Patients 6 and 7 (male brothers) were born from Algerian first-degree cousins (Figure
187 1a). They were lean with normal BMIs and displayed facial acromegalic features,
188 kyphoscoliosis, and lumbar hyperlordosis (Table 1). Both patients presented with walking
189 difficulties at the age of 4 years. Neuromusculoskeletal manifestations included tiptoe walking,
190 absence of deep tendon reflexes, retraction of Achilles tendons and muscle pain. Metabolic
191 features included partial lipoatrophy affecting the shoulder girdle, upper extremities and trunk
192 associated with muscular hypertrophy and pronounced subcutaneous veins, gynecomastia,
193 insulin resistance, acanthosis nigricans, diabetes, liver steatosis, hypertriglyceridemia in the
194 older brother and decreased HDL cholesterol in the younger brother (Figures h, i, Table 1,
195 Supplementary Table 4). Development of secondary sex characteristics was normal, reflected
196 by normal gonadotropin and sex hormone levels (Supplementary Table 4). A demyelinating
197 sensorimotor neuropathy was present in both patients. Brain-MRI revealed Arnold-Chiari
198 malformation type 1 in patient 6 (Supplementary Table 4).

199

200 ***Plaat3*^{-/-} mouse WAT displays a PPAR γ -dependent differentiation defect**

201 The mouse *Plaat3* protein is evolutionary highly conserved and is 83% identical and 91%
202 similar to human PLAAT3 (<https://www.flyrnai.org/diopt>). *Plaat3*^{-/-} mice have been reported
203 to be resistant to diet-induced obesity and show a lipodystrophy syndrome-like phenotype.⁽⁵⁾
204 This phenotype was suggested to be due to loss of PLA₂ activity resulting in a decline in
205 arachidonic acid (AA, C20:4) release and prostaglandin E2 (PGE2) synthesis and an increase
206 in levels of cAMP, leading to increased lipolysis in normally differentiated adipocytes.⁽⁵⁾ Other
207 studies have suggested an important role for PLAAT3 in adipocyte differentiation.⁽¹⁴⁻¹⁶⁾

208 We used *Plaat3*^{-/-} mice-derived inguinal WAT samples⁽¹⁷⁾ for morphological and multi-
209 omics studies to further clarify the pathogenic mechanism of PLAAT3-related lipodystrophy
210 syndrome. We collected 12 inguinal *Plaat3*^{-/-} WAT biopsy specimens and 12 *Plaat3*^{+/-} inguinal
211 WAT control specimens. Light microscopic analysis of *Plaat3*^{-/-} samples and *Plaat3*^{+/-} controls
212 showed that adipocytes in *Plaat3*-deficient tissue were significantly smaller in size, in line with
213 a previous report⁽⁵⁾, while no difference in shape ('circularity') was observed (Figures 3a, b).

214 Next, we evaluated the effect of *Plaat3* deficiency on the lipid content in inguinal WAT
215 using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) lipidomics approach.
216 Dimensionality reduction showed clustering of samples according to sex along the first
217 component and according to genotype (*Plaat3*^{-/-} or *Plaat3*^{+/-}) along the second component,

218 indicating that loss of *Plaat3* activity influences the abundance of certain lipid classes and
219 species (Figure 3c). Given the known enzymatic function of PLAAT3 in membrane
220 phospholipid remodeling, we checked the overall abundance of the 11 phospholipid subclasses
221 in *Plaat3*^{+/+} and *Plaat3*^{-/-} WAT (Supplementary Figure 2). Increased levels were seen for
222 phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and 1-
223 alkenyl, 2-acyl phosphatidylethanolamine (PE-P) in both female and male *Plaat3*^{-/-} WAT, with
224 PI and PS showing the most striking differences (Supplementary Figure 2). Within each
225 phospholipid subclass we filtered all phospholipid species with statistically significant ($p < 0.05$)
226 differences between *Plaat3*^{+/+} and *Plaat3*^{-/-} WAT that showed alterations in the same direction
227 in female and male samples. This revealed a total of 14 lipid species (Supplementary Table 5)
228 of which two, PI(18:0/20:4) and PE(18:0/18:2), represent the most common species within their
229 lipid class and were clearly upregulated in *Plaat3*^{-/-} WAT (Plaat3 mouse lipidomics.html). In
230 animal tissues, PI(18:0/20:4) is the primary source of AA (C20:4) required for biosynthesis of
231 eicosanoids (including prostaglandins) via PLA₂ activity, which releases these bioactive fatty
232 acids from position *sn*-2.⁽¹⁸⁾ However, mediator lipidomics showed no statistically significant
233 differences for AA or AA-derived eicosanoids, including PGE₂, between *Plaat3*^{-/-} and *Plaat3*^{+/+}
234 WAT (Figure 3d) (Plaat3 mouse mediator lipidomics.html).

235 We also performed RNA sequencing on seven *Plaat3*^{-/-} (3 male, 4 female) and eight
236 *Plaat3*^{+/+} mouse (4 male, 4 female) WAT samples. A total of 99 genes were significantly
237 differentially expressed ($|\log_2FC| \geq 1$, raw $p < 0.05$), of which 59 were upregulated and 40
238 downregulated (Figure 3e). We used Metascape to search for enriched pathways within the
239 differentially expressed gene sets (top 100 down- and upregulated genes ranked by FC with raw
240 p -values < 0.05) and found that genes involved in synaptic transmission, glycolipid biosynthetic
241 processes and fat cell differentiation were downregulated, whereas genes involved in
242 myelination and inflammatory responses were upregulated in *Plaat3*^{-/-} WAT (Figure 3f). We
243 used the LISA tool to identify transcriptional regulators (TRs) of gene networks within the
244 down- and upregulated genes (Figure 3g).⁽¹⁹⁾ Interestingly, the most important TR of the
245 downregulated genes was peroxisome proliferator activated receptor gamma (PPARG or
246 PPAR γ), which is responsible for adipocyte differentiation together with its coactivator retinoid
247 X receptor alpha (RXRA or RXR α) with which it forms a heterodimer to induce transcription
248 of target genes.^(20, 21) Transcript levels of the three lipolytic enzymes (*Pnpla2*, *Lipe* and *Mgll*)
249 were not altered in *Plaat3*^{-/-} WAT compared to *Plaat3*^{+/+} (Supplementary Figure 3).

250 Finally, LC-MS/MS-based shotgun proteomics analysis was performed on 9 *Plaat3*^{-/-} (4
251 males, 5 females) and 9 *Plaat3*^{+/+} (4 males, 5 females) inguinal WAT biopsy specimens which

252 reliably quantified a total of 4033 protein groups in all samples. Differential protein intensity
253 analysis ($|\log_2FC| \geq 0.5$, raw $p < 0.05$) showed 41 significantly downregulated and 8 upregulated
254 proteins (Figure 3h). Again, we looked for enriched pathways within the top 100 down- and
255 upregulated proteins (ranked according to FC with raw p-value < 0.05). Proteins involved in
256 fatty acid metabolic and lipid biosynthetic processes were less abundant in *Plaat3^{-/-}* WAT,
257 whereas proteins involved in autophagy and catabolism were more abundant (Figure 3i). With
258 the LISA tool, PPARG and its coactivators, such as CCAAT enhancer binding protein alpha
259 and beta (CEBPA and CEBPB), were again identified as the strongest TRs within the set of
260 downregulated proteins (Figure 3j). The most strongly upregulated protein was Plin2 ($|\log_2FC|$
261 $= 2.69$, raw $p < 0.0001$) (Figure 3h). PLIN2 is known to be expressed on lipid droplet membranes
262 of preadipocytes and is replaced by PLIN1 after completion of the cell maturation process.⁽²²⁾
263 Along with PLIN3, PLIN2 also contributes to the formation of lipid droplet-lysosome contacts
264 and their removal by chaperone-mediated autophagy facilitates lipolysis.^(23, 24) Taken together,
265 these results show that mouse *Plaat3^{-/-}* WAT consists of smaller adipocytes containing
266 increased levels of the PI(18:0/20:4) membrane phospholipid and showing a PPAR γ -dependent
267 adipocyte differentiation defect with abnormal lipid droplet metabolism.

268

269 **Patient WAT shows a PPAR γ -mediated adipogenesis defect**

270 We collected subcutaneous WAT samples of female patients 1 and 4, as well as six healthy
271 female controls with a normal BMI (19-23 kg/m²). Light microscopic analysis showed that
272 patient adipocytes were larger and showed a statistically significant difference in ‘circularity’
273 (Figures 4a, b). Patient WAT also displayed increased inflammation as assessed by CD68
274 staining (Figure 4a (middle panels)). Immunohistochemistry using a PMP70 (70-kDa
275 peroxisomal membrane protein) antibody did not show remarkable differences in peroxisome
276 abundance (Figure 4a (right panels)).

277 LC-MS/MS-based lipidomics analysis was performed on the two patient WAT samples
278 and six controls. Principal component analysis (PCA) based on all the measured lipid species
279 across all classes showed that the lipid profile in the patients clearly differed from that in the
280 controls (Figure 4c). We again focused on phospholipids and observed increased levels for 9 of
281 the 11 phospholipid subclasses (Supplementary Figure 4). Out of 26 phospholipid species with
282 statistically significant ($p < 0.05$) differences between patient and control WAT, 25 were
283 upregulated (Supplementary Table 6). When ranked according to p-value, AA-containing
284 phospholipids LPC(20:4) ($\log_2FC = 2.05 \pm 0.38$, $p < 0.001$) and LPE(20:4) ($\log_2FC = 1.24 \pm 0.28$,
285 $p = 0.004$) were among the top ranked lipid species (Supplementary Table 6, Figure 4d). These

286 results are in line with decreased PLA₂ activity in human PLAAT3-deficient WAT (Figure 4d
287 (right panel)) (PLAAT3 human lipidomics.html).

288 Finally, RNA sequencing was performed on WAT samples from patient 1 and three
289 healthy controls to identify differentially expressed genes as a consequence of *PLAAT3*
290 deficiency. A total of 8711 genes were identified that were significantly up- or downregulated
291 in patient WAT ($|\log_2FC| \geq 1$, raw $p < 0.05$). Of these 8711 genes, the large majority ($n=6710$)
292 were downregulated in patient WAT, whereas 2001 genes were significantly upregulated
293 (Figure 4e). Pathway enrichment analysis of the 100 most downregulated genes (protein coding
294 and noncoding) identified cytoplasmic translation as the most enriched cluster, indicative of a
295 metabolically inactive state of adipocytes. Strikingly, in human WAT, genes involved in the
296 PPAR signaling pathway were also shown to be downregulated (Figure 4f). Transcript levels
297 of well-established PPAR γ target genes involved in adipocyte differentiation (*PLIN1*, *FABP4*),
298 fatty acid transport (*LPL*, *CD36*) and energy homeostasis (*LEP*, *ADIPOQ*) were significantly
299 decreased in patient adipocytes (Figure 4e). The same analysis was performed on the top 100
300 most strongly upregulated genes which identified olfactory transduction and cellular dopamine
301 response as the only significantly upregulated biological pathways. The LISA tool was used to
302 identify TRs of gene networks within the 500 most strongly down- and upregulated genes
303 (Figure 4g). In line with the results in mouse WAT, out of the top 10 ranked TRs of
304 downregulated genes in human WAT, four were strongly linked to PPAR signaling including
305 PPAR γ itself, its two main cooperating TRs CEBPA and CEBPB and its coactivator mediator
306 complex subunit 1 (MED1).^(20, 21, 25) We used Gene Set Enrichment Analysis (GSEA) software
307 to look for enriched gene sets among the differentially expressed genes. Among the
308 downregulated genes, we observed an enrichment of MYC target genes and genes associated
309 with oxidative phosphorylation and adipogenesis (Figures 4h, i). Together, these results are in
310 line with our *Plaat3*^{-/-} mouse data and suggest a PPAR γ -mediated adipogenesis defect
311 accompanied by reduced PLA₂ activity in human PLAAT3-deficient WAT.

312

313 ***PLAAT3* knockout in adipose stem cells disrupts lipid droplet formation**

314 To better address the role of PLAAT3 in human adipogenesis, a custom-designed single-guide
315 RNA (sgRNA)/Cas9 expression vector targeting the second exon of *PLAAT3* was used (Figures
316 5a, b) in human adipose stem cells (ASCs), isolated from abdominal subcutaneous adipose
317 tissue⁽²⁶⁾. Human ASCs were chosen as a model because of their ability to differentiate into
318 mature adipocytes after stimulation *in vitro* (Figure 5c). The efficiency of *PLAAT3* knockout
319 (KO) was confirmed by Sanger sequencing of *PLAAT3* exon 2 in genomic DNA from KO ASC

320 cells, which revealed a high level of on-target indels with 70% insertions and 26% deletions
321 (Figures 5a, b). Control (CTL) cells, expressing scrambled sgRNA, differentiated into
322 adipocytes within 20 days (D20) (Figures 5c, d) and displayed strong accumulation of lipid
323 droplets and TG content in the cytoplasm (Figures 5e, f). In contrast, *PLAAT3* KO led to a
324 strong and significant decrease in lipid droplet formation ($p < 0.0001$) (Figures 5d, e) as well
325 as TG content ($p < 0.0001$) (Figure 5f). The perilipin level, encoded by *PLIN1*, a mature
326 adipocyte marker, was sharply decreased in *PLAAT3* KO cells (Figure 5g). Interestingly, the
327 *PLAAT3* KO cells displayed decreased expression of PPAR γ (Figure 5g). We next investigated
328 the effect of PLAAT3 loss on insulin sensitivity. In WT and control pre-adipocytes (D0)
329 stimulated with insulin, Western blot analysis revealed a strong increase in the phosphorylation
330 of AKT and extracellular-regulated kinase (ERK) (Figure 5h). In contrast, the *PLAAT3* KO
331 cells at D0 were resistant to insulin, as shown by the lack or strong decrease in the
332 phosphorylation of these intermediates upon insulin stimulation (Figure 5h). Altogether, these
333 findings confirm that PLAAT3 regulates adipocyte differentiation through a cellular process
334 involving PPAR γ .

335

336 **DISCUSSION**

337 This study establishes PLAAT3 deficiency as a monogenic lipodystrophy syndrome associated
338 with neurological manifestations. Our data suggest a key pathogenic role for PLAAT3 in
339 PPAR γ -mediated adipogenesis and thus identify PLAAT3 as a potential therapeutic handle to
340 regulate PPAR γ signaling, a promising therapeutic target to increase insulin sensitivity in type
341 2 diabetes.⁽²⁷⁾

342 We provide genetic evidence for the involvement of *PLAAT3* in a complex metabolic
343 and neurological syndrome. We report seven patients from four unrelated families harboring
344 different bi-allelic loss-of-function variants in *PLAAT3* and displaying a clinical presentation
345 combining lipodystrophy syndrome with neurological features, consistent with the expression
346 profile of *PLAAT3*, which is mainly expressed in adipose tissue, brain, and peripheral nerves
347 (<https://www.gtexportal.org/home/gene/PLA2G16>). In all patients, various degrees of
348 lipoatrophy were seen, associated with the metabolic features secondary to the loss of healthy
349 adipose tissue. The historical classification of lipodystrophy syndrome distinguishes the
350 generalized forms (congenital generalized lipodystrophy - CGL) with generalized fat loss
351 apparent at birth and the partial forms (familial partial lipodystrophy - FPLD) beginning later

352 in life, frequently in adolescence. However, this dichotomy does not apply to all situations, and
353 some patients with genetic lipodystrophy syndrome have already been reported with normal fat
354 distribution at birth and the appearance of generalized fat loss later in life.^(28, 29) In patients with
355 *PLAAT3* deficiency, both partial lipoatrophy associated with facio-cervical lipohypertrophy and
356 generalized lipoatrophy are observed. Bi-allelic mutations in *PCYT1A* represent another
357 example of an unclassified genetic lipodystrophy syndrome that can present as either
358 generalized or partial fat loss.^(30, 31) All investigated *PLAAT3*-deficient patients displayed
359 common complications associated with lipodystrophy syndrome (e.g. insulin resistance,
360 diabetes, hypertriglyceridemia, liver steatosis, and polycystic ovary syndrome). Most patients
361 also presented with neurological features. Demyelinating sensorimotor neuropathy was present
362 in all investigated patients. Psychomotor retardation or intellectual disability was present in half
363 of them.

364 Using multi-omics studies in human and murine WAT, we discovered a downregulated
365 PPAR γ -mediated gene network as a downstream consequence of *PLAAT3* inactivation.
366 PPAR γ is the master regulator of adipocyte differentiation and function and was identified as
367 the causal gene for an autosomal dominant lipodystrophy syndrome called FPLD3.⁽³²⁾
368 Accordingly, our study, using CRISPR-Cas9 KO of *PLAAT3* in human ASCs, revealed an
369 adipocyte differentiation defect characterized by a major decrease in intracellular neutral lipid
370 levels as well as decreased expression of adipogenesis and mature adipocyte markers including
371 PPAR γ . Insulin signaling was also altered, even in pre-adipocytes. These functional data are
372 consistent with the lipoatrophic and insulin-resistant phenotype of *PLAAT3* patients. A similar
373 adipocyte differentiation defect has been reported in other lipodystrophy syndrome of various
374 genetic origins.^(29, 33-35) Our data are in line with earlier studies suggesting a central role for
375 *PLAAT3* in adipocyte differentiation.⁽¹⁴⁻¹⁶⁾ A close link between *PLAAT3* and PPAR γ is
376 supported by a murine adipocyte differentiation study demonstrating that *Plaat3* mRNA levels
377 started to increase between 6 and 12h after Ppar γ -mediated initiation of differentiation.⁽¹⁴⁾ A
378 similar observation was made for human *PLAAT3* transcripts whose expression began to rise
379 6h after induction of human pre-adipocyte differentiation and continued to increase during
380 adipogenesis, like PPAR γ , whose expression started a few hours earlier.⁽¹⁶⁾ The expression of
381 *PLAAT3* is therefore appropriately timed to mediate PPAR γ -driven adipogenesis. The exact
382 mechanisms by which *PLAAT3* and PPAR γ are linked remain however partially elusive. In
383 contrast to our results indicating a disturbed PPAR γ -mediated gene network downstream of
384 *PLAAT3* inactivation, murine *Plaat3* was identified as a direct transcriptional target of PPAR γ ,

385 placing PPAR γ upstream of PLAAT3 in adipogenesis.⁽¹⁴⁾ One explanation could be that, after
386 initial induction of PPAR γ by TFs including C/EBP β and C/EBP δ ,⁽²¹⁾ PPAR γ quickly induces
387 PLAAT3 expression⁽¹⁴⁾, which is then needed to further sustain PPAR γ activity during and
388 possibly after adipocyte differentiation (present study). To date, an endogenous and
389 physiologically relevant ligand for PPAR γ has not been identified but it was shown that
390 naturally occurring bioactive compounds including polyunsaturated fatty acids such as AA
391 efficiently modify PPAR γ activity.^(36, 37) It is thus tempting to speculate that PLAAT3 plays a
392 role as a PPAR γ ligand generator by liberating AA or related bioactive lipids from membrane
393 phospholipids in WAT. Although the increased levels of AA-containing (lyso)phospholipids in
394 murine and human PLAAT3-deficient WAT observed in the lipidomics studies would support
395 this hypothesis, we could not detect significantly altered free AA levels or other bioactive lipid
396 mediators in murine *Plaat3*-deficient WAT. Further research is needed to explore this
397 possibility.

398 Furthermore, it cannot be excluded that PLAAT3 plays an additional PPAR γ -dependent
399 role in mature adipocytes. Indeed, next to its central involvement in adipogenesis, PPAR γ is
400 crucial for mature fat cell function where it regulates lipid metabolism and glucose
401 homeostasis.⁽²⁷⁾ In line with this idea, a previous report in mice has shown chronically
402 upregulated lipolysis in normally differentiated *Plaat3*^{-/-} adipocytes due to decreased PGE2
403 levels as a consequence of abrogated PLA₂ activity, which is a known inhibitor of lipolysis.^{(5,}
404 ³⁸⁾ However, our mediator lipidomics study could not confirm the reduced PGE2 levels in
405 murine *Plaat3*-deficient WAT and no upregulation of lipolytic enzymes on the transcript or
406 protein level were seen. Nevertheless, we did not formally investigate lipolysis in the current
407 study, and it is possible that it occurs in human *PLAAT3* patients. Similar observations have
408 been made for the *LIPE* gene involved in another form of lipodystrophy syndrome.⁽³⁹⁾

409 Comparison of *Plaat3* null mice described in 2009 with patients carrying homozygous
410 pathogenic *PLAAT3* variants reveals a number of similarities: a lipoatrophic phenotype,
411 decreased circulating levels of leptin and adiponectin, insulin resistance and liver steatosis.⁽⁵⁾
412 Nevertheless, some differences can also be noted. Serum TG were lower in KO mice, whereas
413 most patients display hypertriglyceridemia. Mice display smaller adipocytes (current study and
414 previous data), whereas adipocyte size in patient WAT seems to be slightly increased.⁽⁵⁾ Such
415 discrepancies are not unusual and, while there is no doubt on the usefulness of mouse models
416 to study human LS, differences between human and murine fat distribution and lipid
417 metabolism represent known limitations.^(40, 41)

418

419 Due to early rodent studies suggesting potential antiobesity and antiviral properties,
420 modifying PLAAT3 activity was thought to be an interesting therapeutic strategy. Interestingly,
421 while it was shown that drug targets with human genetic support are twice as likely to lead to
422 approved drugs^(42,43), our study suggests that blocking PLAAT3 activity too strongly could lead
423 to unwanted metabolic side-effects secondary to a downregulation of PPAR γ -regulated gene
424 networks.

425

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456 **Author Contributions Statement**

457 N.S., D.H., J.G., S.E.C., C.D., I.J. and B.D. wrote the manuscript. N.S., J.G., I.J. and B.D.
458 designed the study and performed the main analyses. N.S., S.E.C., D.H., M.V., S.N., M.T.,
459 C.V., N.V.D., F.R.C., J.A.U., J.C., W.T., and S.C. were involved in phenotyping and clinical
460 follow-up of the patients. N.S., E.B., T.R., G.D., E.D., B.F., F.A., S.K., P.H., C.D., B.P., I.J.
461 and B.D. were involved in genotyping of the patients. S.D. and F.I. performed the proteomics
462 analysis. J.G. designed the CRISPR-Cas9-mediated PLAAT3 knockout cellular model. M.A.
463 provided technical support for the cell experiments. N.S. and B.D. collected adipose tissue
464 biopsy specimens. F.O. and N.M. provided WAT from *Plaat3^{-/-}* and *Plaat3^{+/-}* mice. J.V.D. and
465 C.V.H. performed histological analyses of human and mouse WAT. N.S., D.H., J.G., I.J. and
466 B.D. edited the manuscript.

467

468 **Competing Interests Statement**

469 The authors declare no competing interests.

470 **Tables**471 **Table 1. Clinical and biological characteristics of patients with biallelic truncating *PLAAT3* variants.**

| | Family 1: Patient 1 | Family 1: Patient 2 | Family 1: Patient 3 | Family 2: Patient 4 | Family 3: Patient 5 | Family 4: Patient 6 | Family 4: Patient 7 | Summary |
|---|---|---|---|-------------------------------|------------------------|------------------------|------------------------|----------------------------------|
| Homozygous <i>PLAAT3</i> variant | c.16-4823_118+167del p.(Pro6ValfsTer15) | c.16-4823_118+167del p.(Pro6ValfsTer15) | c.16-4823_118+167del p.(Pro6ValfsTer15) | c.286dupG p.(Ala96GlyfsTer16) | c.339C>A p.(Cys113Ter) | c.339C>A p.(Cys113Ter) | c.339C>A p.(Cys113Ter) | |
| Sex | female | female | female | female | male | male | male | female (4/7), male (3/7) |
| Age at diagnosis (y) | 38 | 37 | 25 | 42 | 2 | 15 | 11 | mean: 27 |
| Age at symptom onset (y) | | | | | | | | |
| - Metabolic features | 33 | n.a. | 18 | 14 | 4 | n.a. | n.a. | |
| - Neurological/skeletal features | 19 | n.a. | 8 | 11 | 1 | 4 | 4 | |
| LS features | | | | | | | | |
| Lipoatrophy | generalized | partial | partial | partial | generalized | partial | partial | partial (5/7), generalized (2/7) |
| Body-mass index (kg/m ²) | 19.7 | 20.5 | 20.5 | 24.6 | 20.7 | 22.2 | 19.1 | mean: 21 |
| Insulin resistance | + | + | + | + | + | + | + | 7/7 |
| Liver steatosis | + | + | + | + | + | + | + | 7/7 |
| Dyslipidemia/hypertriglyceridemia | + | + | + | + | - | + | + | 6/7 |
| Neurological features | | | | | | | | |
| Psychomotor retardation/intellectual disability | - | + | - | - | + | + | - | 3/7 |
| Demyelinating peripheral neuropathy | + | n.a. | n.a. | + | + | + | + | 5/7 |
| Musculoskeletal features | | | | | | | | |

| | | | | | | | | |
|----------------------------|-------------------------|------|-------------------------|------|--|-----------------------------------|-----------------------------------|-----|
| Kyphoscoliosis | - | - | - | - | + | + | + | 3/7 |
| Chronic muscle/joint pain | + | n.a. | + | + | - | + | + | 4/7 |
| Acromegalic features | + | - | + | - | - | + | + | 4/7 |
| Additional features | | | | | | | | |
| Gynecomastia | n.a. | n.a. | n.a. | n.a. | + | + | + | 3/3 |
| Dysmorphic features | prominent nose and chin | - | prominent nose and chin | - | low set ears, deep set eyes, prognathism, beaked nose, flat forehead | short neck, low hair implantation | short neck, low hair implantation | 5/7 |

472 n.a.: not available/not applicable

473 **FIGURE LEGENDS**

474

475 **Figure 1. Family pedigree structures, genetic findings and schematic display of the**
476 ***PLAAT3* null variants in families 1, 2, 3 and 4.**

477 (a) Pedigrees of families 1, 2, 3 and 4. Affected family members are shown with filled circles
478 (females) or squares (males). Diamond-shaped symbols are used when sex is unspecified.
479 Arrows indicate the index patients. Double horizontal lines indicate consanguinity, diagonal
480 lines are used for deceased individuals. Genotypes, if known, are depicted in the pedigrees (-/-
481 for homozygotes, +/- for heterozygotes). Arrows point to the index patient in the pedigree. (b)
482 *Left panel.* Integrative Genome Viewer (IGV) screenshots showing whole exome sequencing
483 (WES) (upper left panel) and whole genome sequencing (WGS) (lower left panel) reads mapped
484 to the reference *PLAAT3* sequence. No reads are mapped to exon 2 of the *PLAAT3* gene in
485 patients 1, 2 and 3, indicative of a homozygous deletion. *Right panel.* Sanger sequencing of the
486 homozygous single nucleotide insertion in patient 4 of family 2 and the nonsense variant in
487 patients 5, 6 and 7 in families 3 and 4. The variants are indicated with an arrow. (c) Schematic
488 overview of the *PLAAT3* homozygous null variants identified through WES/WGS in families
489 1,2, 3 and 4: the deletion of exon 2 (indicated in red) of the *PLAAT3* gene in family 1, the single
490 base insertion in exon 3 in family 2, and the nonsense variant in exon 3 in families 3 and 4
491 (upper panel). The consequences of the molecular defects are also shown at the mRNA level
492 (middle panel) and at the protein level (lower panel). The enzymatically active LRAT and a
493 transmembrane region (TM) of *PLAAT3* are depicted. All three variants result in a premature
494 termination codon and are predicted to cause NMD.

495

496 **Figure 2. Dual X-ray absorptiometry scan and clinical pictures of *PLAAT3*-deficient**
497 **patients.**

498 (a) Dual Energy X-ray Absorptiometry (DEXA) scan of patient 1 showing a lipoatrophic body
499 composition with a total fat percentage of 13.8%. (b) Lateral view of patient 1 showing
500 muscular hypertrophy in the upper and lower limbs. (c) Frontal view of patient 4 demonstrating
501 masculine features with muscle hypertrophy and lipoatrophy of the upper and lower limbs with
502 submental accumulation of adipose tissue. (d) Frontal view of patient 5 showing generalized
503 lipodystrophy, gynecomastia, *genu valgum* and muscle hypertrophy of the upper limbs. (e)
504 Lateral view of patient 5 showing gynecomastia. (f) Dorsal view of patient 5 demonstrating
505 thoracic hyperkyphosis. (g) Foot deformities in patient 5 including *pes cavus* and hammer toes.

506 (h) Frontal view of patient 6 showing lipoatrophy of the trunk and gynecomastia. (i) *Acanthosis*
507 *nigricans* in the axillary region of patient 6.

508

509 **Figure 3. Histopathology, lipidomics, proteomics and differentially expressed gene**
510 **analysis in *Plaat3*^{-/-} and *Plaat3*^{+/-} mouse WAT.**

511 (a) Light microscopic images after staining with hematoxylin and eosin (H&E) of inguinal
512 white adipose tissue (WAT) from a *Plaat3*^{+/-} and a *Plaat3*^{-/-} male mouse. Scale bar represents
513 200 μm . (b) Box plot of adipocyte size measurement (μm^2) ($p < 0.001$) (left panel) and
514 morphology expressed as ‘circularity’ (0-1) (right panel) of *Plaat3*^{+/-} and *Plaat3*^{-/-} mouse WAT.
515 The center line represents the median, the box limits represent the interquartile range (IQR) and
516 the whiskers indicate the minimum and maximum values. p -values were calculated using two-
517 sided Independent-Samples T test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (c) Principal component
518 analysis (PCA) of lipidomics on WAT from male (n=6) and female (n=6) *Plaat3*^{-/-} and male
519 (n=6) and female (n=6) *Plaat3*^{+/-} mice. (d) Bar charts of phosphatidyl inositol (PI) (18:0/20:4)
520 (male: raw $p = 0.008$, female: raw $p = 0.007$), phosphatidyl ethanolamine (PE) (18:0/18:2) (male:
521 raw $p = 0.003$, female: raw $p = 0.004$), arachidonic acid (AA) and prostaglandin E2 (PGE2)
522 levels depicted as the means \pm standard error of the mean (SEM). p -values were calculated
523 using one-way analysis of variance (ANOVA), and the Benjamini-Hochberg procedure was
524 conducted to correct for multiple testing. (e) Volcano plot showing down- (blue) and
525 upregulated (yellow) differentially expressed genes (DEGs) with $|\text{FC}| \geq 2$ and raw $p < 0.05$
526 (ExactTest in edgeR) in WAT from *Plaat3*-deficient male (n=3) and female (n=4) mice
527 compared to male (n=4) and female (n=4) *Plaat3*^{+/-} mice. DEGs above the red line: false
528 discovery rate (FDR)-adjusted $p < 0.05$ (Benjamini-Hochberg correction). (f) Kyoto
529 Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) pathway enrichment
530 analysis of DEGs (top 100 down- (blue) and upregulated (yellow) genes ranked according to
531 FC with raw $p < 0.05$) using Metascape (hypergeometric test and Benjamini-Hochberg
532 correction). (g) Top 10 transcriptional regulators (TRs) of the down- (blue) and upregulated
533 (yellow) genes (top 100 ranked according to FC with raw $p < 0.05$) identified by LISA (one-
534 sided Wilcoxon rank-sum test is used to assess statistical significance). (h) Volcano plot
535 showing down- (blue) and upregulated (yellow) proteins with differential protein intensities
536 with $|\text{FC}| \geq 1.4$ and raw $p < 0.05$ (two-sided Independent-Samples T test) in WAT from male
537 (n=4) and female (n=5) *Plaat3*^{-/-} and male (n=4) compared to female (n=5) *Plaat3*^{+/-} mice.
538 Proteins above the red line: FDR-adjusted $p < 0.05$ (Benjamini-Hochberg correction). (i) KEGG
539 and GO pathway enrichment analysis of differentially abundant proteins (top 100 down- (blue)

540 and upregulated (yellow) ranked according to FC with raw $p < 0.05$) using Metascape. **(j)** Top
541 10 TRs of down- (blue) and upregulated (yellow) proteins (top 100 ranked according to FC with
542 raw $p < 0.05$) identified using LISA.

543

544 **Figure 4. Histopathology, lipidomics and differentially expressed gene analysis of patient**
545 **and control WAT biopsies.**

546 Data were obtained from upper arm subcutaneous WAT of patients 1 and 4, as well as 6 control
547 subjects (n=8). **(a)** Light microscopic images after H&E, CD68 and PMP70 staining of control
548 and patient (p1) WAT. Scale bar represents 200 μm . **(b)** Box plot of adipocyte size measurement
549 (μm^2) ($p < 0.001$) (upper panel) and morphology expressed as ‘circularity’ (0-1) ($p = 0.003$)
550 (lower panel) patient and control WAT. The center line represents the media, the box limits
551 represent the interquartile range (IQR) and the whiskers indicate the minimum and maximum
552 values. p -values were calculated using two-sided Independent-Samples T Test. $*p < 0.05$,
553 $**p < 0.01$, $***p < 0.001$. **(c)** PCA of lipidomics in two patient samples (in purple) and six
554 control samples (in green). **(d)** Bar charts of PI (18:0/20:4), lysophosphatidylcholine (LPC)
555 (20:4) (raw $p < 0.001$, FDR adjusted $p = 0.017$) and lysophosphatidylethanolamine (LPE) (20:4)
556 (raw $p = 0.004$, FDR adjusted $p = 0.044$) levels expressed as the means \pm SEM. p -values were
557 calculated using one-way ANOVA, and the Benjamini-Hochberg procedure was conducted to
558 correct for multiple testing. **(e)** Volcano plot showing the down- (blue) and upregulated (yellow)
559 DEGs with $|\text{FC}| \geq 2$ and raw $p < 0.05$ (ExactTest in edgeR) in WAT from patient 1 compared to
560 three controls (n=4). DEGs above the red line: FDR-adjusted $p < 0.05$ (Benjamini-Hochberg
561 correction). **(f)** KEGG and GO pathway enrichment analysis of DEGs (top 100 down- (blue)
562 and upregulated (yellow) genes ranked according to FC with raw $p < 0.05$) using Metascape
563 (hypergeometric test and Benjamini-Hochberg correction). **(g)** Top 10 TRs of down- (blue) and
564 upregulated (yellow) genes (top 500 ranked according to FC with raw $p < 0.05$) identified using
565 LISA (one-sided Wilcoxon rank-sum test is used to assess statistical significance). **(h)** Top 10
566 enriched hallmark gene sets within the downregulated genes identified using Gene Set
567 Enrichment Analysis (GSEA) software, ranked according to the normalized enrichment score
568 (NES). **(i)** Enrichment plots with NES of the top 3 enriched hallmark gene sets.

569

570 **Figure 5. PLAAT3 deficiency suppresses white adipocyte differentiation of ASCs.**

571 Data were obtained from human adipose stem cells (ASCs), ASCs with a CRISPR-Cas9-
572 mediated PLAAT3-knockout (KO), and ASCs transduced with a control (CTL) Cas9/scramble
573 gRNA plasmid. p -values were determined by one-way ANOVA with the Geisser-Greenhouse

574 correction and Tukey's multiple comparisons test. The results are expressed as means \pm SEM,
575 with $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, $****p < 0.0001$. **(a)** Sanger sequencing confirmed
576 high recombination in the target region (exon 2). The expected break site is indicated by a
577 vertical dotted line and the gRNA sequence is underlined. **(b)** The CRISPR indel pattern
578 analysis in CTL and edited ASCs using Synthego software. The discordance plot showed a high
579 rate of sequence misalignment after the gRNA target site, validating an editing efficiency of
580 96%. **(c)** A timeline representation of ASC differentiation with a hormonal cocktail. IBMX: 3-
581 isobutyl-1-methylxanthine; D0: day 0; D10: day 10; D20: day 20. Scale bar represents 100 μm .
582 **(d)** Adipocyte differentiation was assessed through Oil Red-O lipid staining, with representative
583 images shown by optical and fluorescence microscopy. Scale bar represents 100 μm . **(e)**
584 Quantification of Oil Red-O fluorescence normalized to DNA content (DAPI). The
585 measurements represent three independent experiments (6 replicates each) ($p < 0.0001$). Light
586 grey bars correspond to control conditions (ASC, CTL), dark grey bars correspond to knockout
587 (KO) condition. **(f)** Intracellular triglyceride content at D20. The measurements represent four
588 independent experiments. ($p < 0.0001$). Light grey bars correspond to control conditions (ASC,
589 CTL), dark grey bars correspond to knockout (KO) condition. **(g)** Western blot analysis of
590 adipocyte markers at D20 in ASC cells. Images are representative of three independent
591 experiments. Quantification was performed using FIJI software and normalized to the value of
592 CTL cells at D20. PPAR γ : KO vs ASC $p=0.0010$, KO vs CTL $p=0.0015$. Perilipin: KO vs ASC
593 $p=0.0113$, KO vs CTL $p=0.0165$. **(h)** Activation of insulin signaling in ASCs before
594 differentiation at D0. ASC, CTL and PLAAT3 KO cells were deprived of serum for 6 hr,
595 stimulated with 20 nM insulin for 5 min or left untreated, and subjected to immunoblotting with
596 antibodies against total and phospho-AKT, and extracellular-regulated kinase (ERK)1/2.
597 Western blot images are representative of three independent experiments and were quantified
598 using FIJI software, with normalization to CTL cells at D20 treated with insulin. pAKT: KO vs
599 CTL $p=0.0239$. pERK: KO vs ASC $p=0.00471$, KO vs CTL $p=0.0103$.

600

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602

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604 **References**

- 605 1. Pang XY, Cao J, Addington L, Lovell S, Battaile KP, Zhang N, et al. Structure/function
606 relationships of adipose phospholipase A2 containing a cys-his-his catalytic triad. *J Biol Chem.*
607 2012;287(42):35260-74.
- 608 2. Duncan RE, Sarkadi-Nagy E, Jaworski K, Ahmadian M, Sul HS. Identification and functional
609 characterization of adipose-specific phospholipase A2 (AdPLA). *J Biol Chem.* 2008;283(37):25428-36.
- 610 3. Uyama T, Jin XH, Tsuboi K, Tonai T, Ueda N. Characterization of the human tumor suppressors
611 TIG3 and HRASLS2 as phospholipid-metabolizing enzymes. *Biochim Biophys Acta.* 2009;1791(12):1114-
612 24.
- 613 4. Uyama T, Morishita J, Jin XH, Okamoto Y, Tsuboi K, Ueda N. The tumor suppressor gene H-
614 Rev107 functions as a novel Ca²⁺-independent cytosolic phospholipase A1/2 of the thiol hydrolase
615 type. *J Lipid Res.* 2009;50(4):685-93.
- 616 5. Jaworski K, Ahmadian M, Duncan RE, Sarkadi-Nagy E, Varady KA, Hellerstein MK, et al. AdPLA
617 ablation increases lipolysis and prevents obesity induced by high-fat feeding or leptin deficiency. *Nat*
618 *Med.* 2009;15(2):159-68.
- 619 6. Hussain I, Garg A. Lipodystrophy Syndromes. *Endocrinol Metab Clin North Am.* 2016;45(4):783-
620 97.
- 621 7. Jéru I. Genetics of lipodystrophy syndromes. *Presse Med.* 2021;50(3):104074.
- 622 8. Brown RJ, Araujo-Vilar D, Cheung PT, Dunger D, Garg A, Jack M, et al. The Diagnosis and
623 Management of Lipodystrophy Syndromes: A Multi-Society Practice Guideline. *J Clin Endocrinol Metab.*
624 2016;101(12):4500-11.
- 625 9. Letourneau LR, Greeley SAW. Congenital forms of diabetes: the beta-cell and beyond. *Curr*
626 *Opin Genet Dev.* 2018;50:25-34.
- 627 10. Sollier C, Vazier C, Capel E, Lascols O, Auclair M, Janmaat S, et al. Lipodystrophic syndromes:
628 From diagnosis to treatment. *Ann Endocrinol (Paris).* 2020;81(1):51-60.
- 629 11. Plagnol V, Curtis J, Epstein M, Mok KY, Stebbings E, Grigoriadou S, et al. A robust model for
630 read count data in exome sequencing experiments and implications for copy number variant calling.
631 *Bioinformatics.* 2012;28(21):2747-54.
- 632 12. Raman L, Dheedene A, De Smet M, Van Dorpe J, Menten B. WisecondorX: improved copy
633 number detection for routine shallow whole-genome sequencing. *Nucleic Acids Res.* 2019;47(4):1605-
634 14.
- 635 13. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting
636 investigators with an interest in the same gene. *Hum Mutat.* 2015;36(10):928-30.
- 637 14. Hummasti S, Hong C, Bensinger SJ, Tontonoz P. HRASLS3 is a PPARgamma-selective target gene
638 that promotes adipocyte differentiation. *J Lipid Res.* 2008;49(12):2535-44.
- 639 15. Wang Z, Chai J, Wang Y, Gu Y, Long K, Li M, et al. LncPLAAT3-AS Regulates PLAAT3-Mediated
640 Adipocyte Differentiation and Lipogenesis in Pigs through miR-503-5p. *Genes (Basel).* 2023;14(1).
- 641 16. Michaud A, Lacroix-Pépin N, Pelletier M, Daris M, Biertho L, Fortier MA, et al. Expression of
642 genes related to prostaglandin synthesis or signaling in human subcutaneous and omental adipose
643 tissue: depot differences and modulation by adipogenesis. *Mediators Inflamm.* 2014;2014:451620.
- 644 17. Morishita H, Eguchi T, Tsukamoto S, Sakamaki Y, Takahashi S, Saito C, et al. Organelle
645 degradation in the lens by PLAAT phospholipases. *Nature.* 2021;592(7855):634-8.
- 646 18. Wishart DS, Guo A, Oler E, Wang F, Anjum A, Peters H, et al. HMDB 5.0: the Human
647 Metabolome Database for 2022. *Nucleic Acids Res.* 2022;50(D1):D622-d31.
- 648 19. Qin Q, Fan J, Zheng R, Wan C, Mei S, Wu Q, et al. Lisa: inferring transcriptional regulators
649 through integrative modeling of public chromatin accessibility and CHIP-seq data. *Genome Biol.*
650 2020;21(1):32.
- 651 20. Cataldi S, Costa V, Ciccociola A, Aprile M. PPAR γ and Diabetes: Beyond the Genome and
652 Towards Personalized Medicine. *Curr Diab Rep.* 2021;21(6):18.
- 653 21. Lefterova MI, Haakonsson AK, Lazar MA, Mandrup S. PPAR γ and the global map of
654 adipogenesis and beyond. *Trends Endocrinol Metab.* 2014;25(6):293-302.

- 655 22. Zhang K, Chen X, Zhang P, Liu G. Perilipin2 is an Earlier Marker Than Perilipin1 for Identifying
656 Adipocyte Regeneration in Fat Grafts. *Aesthet Surg J*. 2021;41(6):Np646-np52.
- 657 23. Olzmann JA, Carvalho P. Dynamics and functions of lipid droplets. *Nat Rev Mol Cell Biol*.
658 2019;20(3):137-55.
- 659 24. Kaushik S, Cuervo AM. Degradation of lipid droplet-associated proteins by chaperone-
660 mediated autophagy facilitates lipolysis. *Nat Cell Biol*. 2015;17(6):759-70.
- 661 25. Ge K, Cho YW, Guo H, Hong TB, Guermah M, Ito M, et al. Alternative mechanisms by which
662 mediator subunit MED1/TRAP220 regulates peroxisome proliferator-activated receptor gamma-
663 stimulated adipogenesis and target gene expression. *Mol Cell Biol*. 2008;28(3):1081-91.
- 664 26. Gorwood J, Ejlalmanesh T, Bourgeois C, Mantecon M, Rose C, Atlan M, et al. SIV Infection and
665 the HIV Proteins Tat and Nef Induce Senescence in Adipose Tissue and Human Adipose Stem Cells,
666 Resulting in Adipocyte Dysfunction. *Cells*. 2020;9(4).
- 667 27. Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, et al. PPAR γ signaling and
668 metabolism: the good, the bad and the future. *Nat Med*. 2013;19(5):557-66.
- 669 28. Mory PB, Crispim F, Kasamatsu T, Gabbay MA, Dib SA, Moisés RS. Atypical generalized
670 lipoatrophy and severe insulin resistance due to a heterozygous LMNA p.T10I mutation. *Arq Bras*
671 *Endocrinol Metabol*. 2008;52(8):1252-6.
- 672 29. Gautheron J, Lima L, Akinci B, Zammouri J, Auclair M, Ucar SK, et al. Loss of thymidine
673 phosphorylase activity disrupts adipocyte differentiation and induces insulin-resistant lipoatrophic
674 diabetes. *BMC Med*. 2022;20(1):95.
- 675 30. Payne F, Lim K, Girousse A, Brown RJ, Kory N, Robbins A, et al. Mutations disrupting the
676 Kennedy phosphatidylcholine pathway in humans with congenital lipodystrophy and fatty liver disease.
677 *Proc Natl Acad Sci U S A*. 2014;111(24):8901-6.
- 678 31. Mann JP, Savage DB. What lipodystrophies teach us about the metabolic syndrome. *J Clin*
679 *Invest*. 2019;129(10):4009-21.
- 680 32. Barroso I, Gurnell M, Crowley VE, Agostini M, Schwabe JW, Soos MA, et al. Dominant negative
681 mutations in human PPAR γ associated with severe insulin resistance, diabetes mellitus and
682 hypertension. *Nature*. 1999;402(6764):880-3.
- 683 33. Capel E, Vatier C, Cervera P, Stojkovic T, Disse E, Cottureau AS, et al. MFN2-associated
684 lipomatosis: Clinical spectrum and impact on adipose tissue. *J Clin Lipidol*. 2018;12(6):1420-35.
- 685 34. Sollier C, Capel E, Aguilhon C, Smirnov V, Auclair M, Douillard C, et al. LIPE-related
686 lipodystrophic syndrome: clinical features and disease modeling using adipose stem cells. *Eur J*
687 *Endocrinol*. 2021;184(1):155-68.
- 688 35. Gautheron J, Morisseau C, Chung WK, Zammouri J, Auclair M, Baujat G, et al. EPHX1 mutations
689 cause a lipoatrophic diabetes syndrome due to impaired epoxide hydrolysis and increased cellular
690 senescence. *Elife*. 2021;10.
- 691 36. Hernandez-Quiles M, Broekema MF, Kalkhoven E. PPAR γ in Metabolism, Immunity, and
692 Cancer: Unified and Diverse Mechanisms of Action. *Front Endocrinol (Lausanne)*. 2021;12:624112.
- 693 37. Kim YG, Lou AC, Saghatelian A. A metabolomics strategy for detecting protein-metabolite
694 interactions to identify natural nuclear receptor ligands. *Mol Biosyst*. 2011;7(4):1046-9.
- 695 38. Civelek E, Ozen G. The biological actions of prostanoids in adipose tissue in physiological and
696 pathophysiological conditions. *Prostaglandins Leukot Essent Fatty Acids*. 2022;186:102508.
- 697 39. Hajnal A, Klemenz R, Schäfer R. Subtraction cloning of H-rev107, a gene specifically expressed
698 in H-ras resistant fibroblasts. *Oncogene*. 1994;9(2):479-90.
- 699 40. Rochford JJ. Mouse models of lipodystrophy and their significance in understanding fat
700 regulation. *Curr Top Dev Biol*. 2014;109:53-96.
- 701 41. Le Lay S, Magré J, Prieur X. Not Enough Fat: Mouse Models of Inherited Lipodystrophy. *Front*
702 *Endocrinol (Lausanne)*. 2022;13:785819.
- 703 42. King EA, Davis JW, Degner JF. Are drug targets with genetic support twice as likely to be
704 approved? Revised estimates of the impact of genetic support for drug mechanisms on the probability
705 of drug approval. *PLoS Genet*. 2019;15(12):e1008489.

706 43. Nelson MR, Tipney H, Painter JL, Shen J, Nicoletti P, Shen Y, et al. The support of human genetic
707 evidence for approved drug indications. *Nat Genet.* 2015;47(8):856-60.
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710

711 **METHODS**

712

713 This research complies with all relevant ethical regulation and was approved by the Ethics
714 Committee of the University Hospital of Ghent (EC: 2019/1430). Data collection and analysis
715 were not performed blind to the conditions of the experiment. No animals or data points have
716 been excluded from the analyses.

717

718 **Patient clinical characteristics**

719 All seven patients were clinically evaluated by an endocrinologist and a neurologist. Blood
720 samples were taken to determine the levels of fasting glucose, HbA1c, insulin, cholesterol,
721 triglycerides and leptin. Plasma insulin measurements were performed using the Roche ECLIA
722 technology, and leptin was measured through a radioimmunoassay. Lipoatrophy and regional
723 fat accumulation were objectified using MRI or CT scan imaging. In two patients, dual X-ray
724 absorptiometry was performed to evaluate body composition. In five patients, EMG was
725 performed, and in four patients, brain MRI was performed. In one patient and one control,
726 biopsy specimens of upper arm subcutaneous WAT were obtained for histopathological
727 examination with hematoxylin and eosin and anti-CD68 staining after informed consent of the
728 patient and its caregiver. Quantification of the results was performed using ImageJ software.⁹
729 Statistical analysis was done in SPSS Statistics 27. Written informed consent for multiomics
730 analysis and publication of clinical pictures was obtained from all patients and control
731 individuals. For multi-omics analysis we obtained upper arm subcutaneous WAT biopsies of
732 patients 1 and 4, as well as six healthy, lean, female controls with ages between 25 and 60 years
733 old. The samples size was restricted due to the limited availability of WAT biopsies. No power
734 analysis was performed.

735

736 **Genomics**

737 Genetic studies in family 1 were initiated by the UD-ProZA team at Ghent University Hospital
738 and included SNP-array-based homozygosity mapping, Sanger sequencing and cDNA analysis

739 of *BSCL2*, whole exome sequencing (WES), low-pass shallow whole genome sequencing
740 (CNV-sequencing) and whole genome sequencing (WGS).

741 Homozygosity mapping: Genotyping for homozygosity mapping was performed in three
742 affected and two unaffected members of family 1 using 200K genome-wide HumanCytoSNP-
743 12 v2 BeadChip single nucleotide polymorphism (SNP) arrays (Illumina, San Diego, CA). The
744 position of the probes was based on NCBI build GRCh37. Homozygous regions shared between
745 affected members were detected using the PLINK algorithm (v1.07, default settings).⁽⁴⁴⁾

746 *BSCL2* mutation screening: Sequencing of *BSCL2* was performed using Sanger sequencing
747 after PCR amplification of all exons including splice junctions. Extracted mRNA from cultured
748 lymphocytes was reverse transcribed to cDNA. The region covering *BSCL2* exons 2 to 9 was
749 amplified by PCR from this cDNA. The PCR product was migrated on an agarose gel and its
750 size was compared to that obtained from a control subject.

751 Whole-exome sequencing (WES): WES was performed on the Illumina NovaSeq 6000
752 Platform after enrichment of gDNA with SureSelectXT Low Input Human All Exon v7 (Agilent
753 Technologies). The BWA-MEM 0.7.17 algorithm was used for read mapping against the human
754 genome reference sequence (NCBI, GRCh37.p5/hg19), duplicate read removal, and variant
755 calling. Variant calling and filtering were performed using Seqplorer, an in-house developed
756 tool for the analysis of WES data. The position of the called variants is based on NCBI build
757 GRCh38. A minimum of 90% of the interrogated genes had a coverage of >20x. Variant
758 classification was performed according to the ACMG guidelines⁽⁴⁵⁾ CNVs were detected with
759 ExomeDepth.⁽¹¹⁾

760 Molecular karyotyping was performed by means of low-pass whole-genome sequencing (CNV-
761 seq) on an Illumina NovaSeq 6000 with a genome wide resolution of 100 kb (GRCh38).

762 Whole-genome sequencing was performed by Macrogen on an Illumina NovaSeq 6000
763 platform. Fastq-files were aligned against the hg38 reference genome with BWA-MEM
764 (v0.7.17). Files were converted to BAM format, duplicate marked, and sorted and indexed using
765 Samtools (v1.9)⁽⁴⁶⁾ and Picard (v2.21.6). Structural variants (SVs) were then called using three
766 different callers, namely DELLY (v0.8.3)⁽⁴⁷⁾, LUMPY (v0.2.13)⁽⁴⁸⁾ and Manta (v1.6.0)⁽⁴⁹⁾.
767 Standard settings were used for all.

768 In family 2, genetic analysis was initiated by a clinical geneticist at Hôpital de Hautepierre in
769 Strasbourg and included the analysis of neuropathy and lipodystrophy gene panels, WES and
770 Sanger sequencing. Genetic studies in families 3 and 4 were initiated at Hôpital Pitié-Salpêtrière
771 in Paris and consisted of the analysis of a gene panel including genes involved in lipodystrophic
772 syndromes and WES followed by Sanger sequencing of the region encompassing the *PLAAT3*
773 variant. WES was performed for the index case as part of the ‘Neuromendeliome’ study,
774 including 26 patients with unsolved syndromes affecting the nervous system. Paired-end
775 sequencing libraries were prepared using the Agilent SureSelect XT Human All Exon v7
776 Enrichment kit. Sequencing (2x100 bases) was performed on a HiSeq2500 (Illumina) on the
777 GenomEast platform (IGBMC, Illkirch, France). Image analysis and base calling were
778 performed using CASAVA v1.8.2 (Illumina). Reads were mapped onto the reference genome
779 Hg19 using BWA v0.7.5a.⁽⁵⁰⁾ Elimination of duplicate reads and base quality recalibration were
780 performed using Picard v1.122. Realignment around indels was performed with GATK v3.2-2.
781 Reads mapping to several positions in the genome were excluded using Samtools v0.1.19.⁽⁴⁶⁾
782 Variant calling was done using GATK v3.2-2 Unified genotyper.⁽⁵¹⁾ Variants were annotated
783 using GATK v3.2-2⁽⁵¹⁾, SnpE_ v2.0.5⁽⁵²⁾ and SnpSift v4.4l⁽⁵³⁾.

784

785 **Generation of *Plaat3*^{-/-} mice**

786 All mouse (C57BL/6J) experiments were approved by the Institutional Animal Care and Use
787 Committee of the University of Tokyo (Medical-P17-084) and the Animal Care and Use
788 Committee of the National Institute of Quantum and Radiological Science and Technology
789 (1610111 and 1610121). *Plaat3*^{+/-} mice (C57BL/6J) were intercrossed to obtain *Plaat3*^{-/-}
790 mice.⁽¹⁷⁾ Genotyping of *Plaat3*^{+/-} and *Plaat3*^{-/-} mice was performed by PCR using primers
791 flanking the target site (forward primer, 5'-GAAAGAAGCTGCTGTGCCATGTGGCC-3';
792 reverse primer, 5'-ACCTGATCACTCCGAGGAACTCCATAGC-3') and subsequent
793 acrylamide gel electrophoresis. Wild-type and mutated alleles were detected as 207-bp and 191-
794 bp bands, respectively. 24 inguinal WAT specimens were obtained from 6 male *Plaat3*^{-/-}, 6
795 male *Plaat3*^{+/-}, 6 female *Plaat3*^{-/-}, and 6 female *Plaat3*^{+/-} at the age of 11 weeks. All mice were
796 housed in a specific pathogen-free room maintained at a constant ambient temperature of 20-
797 22°C, 40-60% of humidity under a 12-hour light/dark cycle with free access to water and food.
798 Mice were fed sterilized (30kGy) CLEA Rodent Diet CE-2 standard pellet chow (CLEA Japan,
799 Inc., Tokyo, Japan). No statistical methods were used to pre-determine samples sizes but our

800 sample sizes are similar to those reported in previous publications.^(5, 17) Data collection and
801 analysis were not performed blind to the conditions of the experiments.

802

803 **RNA sequencing**

804 RNA sequencing was performed on 15 inguinal mouse WAT samples. Seven *Plaat3*^{-/-}
805 specimens (3 males, 4 females) were compared to 8 *Plaat3*^{+/-} littermates (4 males, 4 females).
806 Additionally, RNA sequencing was performed on an upper arm WAT sample of patient 1 and
807 three healthy lean controls (females, between 30 and 58 years of age and of European Caucasian
808 descent, BMI ranging between 19 and 22 kg/m²).
809

810 RNA paired-end sequencing was performed by Macrogen on an Illumina platform. Library
811 preparation was performed using the SMARTer Universal Low Input RNA Kit (Takara Bio Inc,
812 #634936) and the TruSeq RNA Sample Prep Kit v2 (Illumina, Inc., #RS-122-2101). Trimmed
813 reads were mapped to the reference genome with HISAT2.⁽⁵⁴⁾ After read mapping, Stringtie
814 was used for transcript assembly.⁽⁵⁵⁾ The expression profile was calculated for each sample and
815 transcript/gene as read count, FPKM (Fragment per Kilobase of transcript per Million mapped
816 reads) and TPM (Transcripts per Kilobase Million). Differentially Expressed Genes (DEG)
817 analysis was performed on a comparison pair (test vs control) using edgeR.⁽⁵⁶⁾ Functional
818 enrichment analysis of KEGG and GO pathways⁽⁵⁷⁾ within 100 most strongly down- and
819 upregulated genes was performed using the Metascape tool.⁽⁵⁸⁾ To identify potential
820 transcriptional regulators (TRs) of the top 100 (mouse WAT) or 500 (human WAT) down- and
821 upregulated genes we performed LISA (epigenetic landscape in silico deletion analysis;
822 <http://lisa.cistrome.org/>), which is designed to combine a comprehensive database of human
823 and mouse DNase-seq, H3K27ac ChIP-seq, and TR Chip-seq to identify TRs that regulate a
824 query gene set.⁽¹⁹⁾

825 **Lipidomics and histopathology**

826 Lipidomics analysis was performed on inguinal WAT of 12 *Plaat3*^{-/-} (6 male, 6 female) and 12
827 *Plaat3*^{+/-} (6 male, 6 female) mice and subcutaneous upper inner arm WAT specimens from two
828 patients (patients 1 and 4) and 6 control individuals (all female, between 27 and 58 years of age
829 and of European Caucasian descent, BMI ranging between 19 and 22 kg/m²) in collaboration
830 with LipometrixTM, the lipidomics core at KU Leuven in Belgium. The subcutaneous adipose

831 tissue specimens were obtained through an open biopsy, and the site of sampling was identical
832 for all patients and controls. After lipid extraction and sample normalization, hydrophilic
833 interaction liquid chromatography-mass spectrometry (HILIC LC-MS/MS) was performed,
834 enabling quantification of 1800 lipid species across 18 different lipid classes
835 (<https://www.lipometrix.be>)

836 Part of the mouse inguinal WAT biopsies were thawed and fixed in 10% neutral-buffered
837 formalin for ~27 hours, paraffin-embedded and stained with hematoxylin and eosin. Light
838 microscopic images were analyzed using ImageJ software.⁹

839 Lipid extraction

840 A sample containing 10 µg of protein was mixed with 800 µl 1 N HCl:CH₃OH 1:8 (v/v), 900
841 µl CHCl₃, 200 µg/ml of the antioxidant 2,6-di-tert-butyl-4-methylphenol (BHT; Sigma Aldrich)
842 and 3 µl of SPLASH® LIPIDOMIX® Mass Spec Standard (#330707, Avanti Polar Lipids).
843 After vortexing and centrifugation, the lower organic fraction was collected and evaporated
844 using a Savant Speedvac spd111v (Thermo Fisher Scientific) at room temperature and the
845 remaining lipid pellet was stored at - 20°C under argon.

846 Mass spectrometry

847 Just before mass spectrometry analysis, lipid pellets were reconstituted in 100% ethanol. Lipid
848 species were analyzed by liquid chromatography electrospray ionization tandem mass
849 spectrometry (LC-ESI/MS/MS) on a Nexera X2 UHPLC system (Shimadzu) coupled with a
850 hybrid triple quadrupole/linear ion trap mass spectrometer (6500+ QTRAP system; AB
851 SCIEX). Chromatographic separation was performed on an XBridge amide column (150 mm ×
852 4.6 mm, 3.5 µm; Waters) maintained at 35°C using mobile phase A [1 mM ammonium acetate
853 in water-acetonitrile 5:95 (v/v)] and mobile phase B [1 mM ammonium acetate in water-
854 acetonitrile 50:50 (v/v)] in the following gradient: (0-6 min: 0% B → 6% B; 6-10 min: 6% B
855 → 25% B; 10-11 min: 25% B → 98% B; 11-13 min: 98% B → 100% B; 13-19 min: 100% B;
856 19-24 min: 0% B) at a flow rate of 0.7 mL/min which was increased to 1.5 mL/min from 13
857 minutes onwards. SM, CE, CER, DCER, HCER and LCER were measured in positive ion mode
858 with precursor scans of 184.1, 369.4, 264.4, 266.4, 264.4 and 264.4 respectively. TAG, DAG
859 and MAG were measured in positive ion mode with a neutral loss scan for one of the fatty acyl
860 moieties. PC, LPC, PE, LPE, PG, PI and PS were measured in negative ion mode by fatty acyl
861 fragment ions. Lipid quantification was performed by scheduled multiple reaction monitoring
862 (MRM), and the transitions were based on neutral losses or typical product ions as described

863 above. The instrument parameters were as follows: curtain gas = 35 psi; collision gas = 8 a.u.
864 (medium); ion spray voltage = 5500 V and -4,500 V; temperature = 550°C; ion source gas 1 =
865 50 psi; ion source gas 2 = 60 psi; declustering potential = 60 V and -80 V; entry potential = 10
866 V and -10 V; collision cell exit potential = 15 V and -15 V.

867 The following fatty acyl moieties were taken into account for the lipidomic analysis: 14:0, 14:1,
868 16:0, 16:1, 16:2, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 20:2, 20:3, 20:4, 20:5, 22:0, 22:1, 22:2, 22:4,
869 22:5 and 22:6, except for TGs, which considered 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:3, 20:4,
870 20:5, 22:2, 22:3, 22:4, 22:5, and 22:6.

871 Data Analysis

872 Peak integration was performed with the MultiQuant™ software version 3.0.3. Lipid species
873 signals were corrected for isotopic contributions (calculated with Python Molmass 2019.1.1)
874 and were quantified based on internal standard signals and adheres to the guidelines of the
875 Lipidomics Standards Initiative (LSI) (level 2 type quantification as defined by the LSI).
876 Unpaired T-test p-values and FDR corrected p-values (using the Benjamini/Hochberg
877 procedure) were calculated in Python StatsModels version 0.10.1.

878 **Proteomics**

879 Sample preparation

880 Proteomics analysis was performed on inguinal WAT of 9 *Plaat3*^{-/-} (4 male, 5 female) and 9
881 *Plaat3*^{+/-} (4 male, 5 female) mice. Inguinal WAT tissue in 1.5 ml tubes was mechanically
882 disrupted by 2 cycles of grinding with a disposable micropestle and freeze-thawing. Then, 250
883 µl lysis buffer containing 5% sodium dodecyl sulfate (SDS) and 50 mM triethylammonium
884 bicarbonate (TEAB), pH 8.5, was added to the tissue, and the samples were ground once more
885 with a micropestle to disrupt any remaining intact material. The tubes were spun down shortly,
886 each sample was divided over 3 wells of a PIXUL™ 96-well plate (Active Motif) and samples
887 were sonicated with a PIXUL™ Multisample sonicator (Active Motif) for 30 minutes with
888 default settings (Pulse 50 cycles, PRF 1 kHz, Burst Rate 20 Hz). After centrifugation for 5 min
889 at 2204 x g at room temperature (RT), the bottom (non-lipid) layer was transferred to new tubes
890 and the protein concentration was measured by bicinchoninic acid (BCA) assay (Thermo
891 Scientific). From each sample, 100 µg of protein was isolated to continue the protocol. Proteins
892 were reduced by the addition of 15 mM dithiothreitol and incubation for 30 minutes at 55°C

893 and then alkylated by the addition of 30 mM iodoacetamide and incubation for 15 minutes at
894 RT in the dark. Phosphoric acid was added to a final concentration of 1.2%, and subsequently,
895 samples were diluted 7-fold with binding buffer containing 90% methanol in 100 mM TEAB,
896 pH 7.55. The samples were loaded on a 96-well S-Trap™ plate (Protifi), placed on top of a
897 deepwell plate, and centrifuged for 2 min at 1,500 x g at RT. After protein binding, the S-trap™
898 plate was washed three times by adding 200 µl binding buffer and centrifugation for 2 min at
899 1,500 x g at RT. A new deepwell receiver plate was placed below the 96-well S-Trap™ plate
900 and 50 mM TEAB containing trypsin (1/100, w/w) was added for digestion overnight at 37°C.
901 Using centrifugation for 2 min at 1,500 x g, peptides were eluted three times, first with 80 µl
902 50 mM TEAB, then with 80 µl 0.2% formic acid (FA) in water and finally with 80 µl 0.2% FA
903 in water/acetonitrile (ACN) (50/50, v/v). Eluted peptides were dried completely by vacuum
904 centrifugation.

905 TMTpro™ 18-plex labels (0.5 mg, Thermo Fisher Scientific) were equilibrated to RT
906 immediately before use and dissolved in 20 µl anhydrous acetonitrile (ACN). The dried peptides
907 were resuspended in 80 µl 100 mM TEAB (pH 8.5), the peptide concentration was determined
908 on a Lunatic spectrophotometer (Unchained Labs)⁽⁵⁹⁾ and the peptide amount was adjusted to
909 40 µg for each sample. Peptides were labeled for 1 hour at RT using 0.5 mg of TMTPro™ label
910 (labels used: 135N, 132C, 131N, 132N, 134N, 133C, 133N, 134C, 131C: *Plaat3*^{+/-}
911 heterozygous replicates; 130C, 128N, 126C, 127C, 129C, 129N, 128C, 130N, 127N: *Plaat3*^{-/-}
912 homozygous replicates). The reaction was quenched for 15 min at RT by the addition of 4.2 µl
913 of 5% hydroxylamine. The 18 labeled samples were combined, and 100 µg labeled peptides
914 were isolated, dried by vacuum centrifugation, redissolved in 100 µl 0.1% TFA and desalted on
915 a reversed phase (RP) C18 OMIX tip (Agilent). The tip was first washed 3 times with 100 µl
916 pre-wash buffer (0.1% TFA in water/ ACN (20:80, v/v)) and pre-equilibrated 5 times with 100
917 µl of wash buffer (0.1% TFA in water) before the sample was loaded on the tip. After peptide
918 binding, the tip was washed 3 times with 100 µl of wash buffer, and peptides were eluted twice
919 with 100 µl elution buffer (0.1% TFA in water/ACN (40:60, v/v)). The combined elutions were
920 dried in a vacuum concentrator.

921 Vacuum-dried peptides were redissolved in 100 µl loading solvent A (0.1% TFA in water/ACN
922 (98:2, v/v)), and 95 µl was injected for fractionation by RP-HPLC (Agilent series 1200)
923 connected to a Probot fractionator (LC Packings). Peptides were first loaded in solvent A on a
924 4 cm precolumn (made in-house, 250 µm internal diameter (ID), 5 µm C18 beads, Dr. Maisch)
925 for 10 min at 25 µl/min and then separated on a 15 cm analytical column (made in-house, 250

926 μm ID, 3 μm C18 beads, Dr Maisch). Elution was performed using a linear gradient from 100%
927 RP-HPLC solvent A (10 mM ammonium acetate (pH 5.5) in water/ACN (98:2, v/v)) to 100%
928 RP-HPLC solvent B (70% ACN, 10 mM ammonium acetate (pH 5.5)) in 100 min at a constant
929 flow rate of 3 $\mu\text{L}/\text{min}$. Fractions were collected every minute between 20 and 92 minutes and
930 pooled every 24 minutes to generate a total of 24 samples for LC-MS/MS analysis. All 24
931 fractions were dried under vacuum in HPLC inserts and stored at -20°C until further use.

932

933 LC-MS/MS analysis

934 Each fraction was solubilized in 20 μL loading solvent A (0.1% TFA in water:ACN (98:2, v:v))
935 moments before analysis. 15 μl of the sample measured on Dropsense16 (Unchained Labs) was
936 injected for LC-MS/MS analysis with an Ultimate 3000 RSLCnano system in-line connected
937 to an Orbitrap Fusion Lumos mass spectrometer (Thermo). Trapping was performed at 20
938 $\mu\text{l}/\text{min}$ for 2 min in loading solvent A on a 20 mm trapping column (Thermo Scientific, 300 μm
939 internal diameter (I.D.), 5 μm beads). The peptides were separated on a 110 cm prototype
940 $\mu\text{PAC}^{\text{TM}}$ column (Thermo Scientific). It was kept at a constant temperature of 50°C . Peptides
941 were eluted by a linear gradient reaching 26.4% MS solvent B (0.1% FA in water/acetonitrile
942 (2:8, v/v)) after 45 min, 44% MS solvent B at 55 min, 56% MS solvent B at 60 min, followed
943 by a 10-min wash at 56% MS solvent B and re-equilibration with MS solvent A (0.1% FA in
944 water). In the first 15 min, the flow rate was set to 600 nl/min after which it was kept constant
945 at 300 nl/min .

946 The mass spectrometer was operated in data-dependent mode. Full-scan MS spectra (375-1500
947 m/z) were acquired at a resolution of 120,000 in the Orbitrap analyzer after accumulation to a
948 target AGC value of 400,000 with a maximum injection time of 50 ms. The precursor ions were
949 filtered for charge states (2-7 required), dynamic exclusion (60 s; \pm 10 ppm window) and
950 intensity (minimal intensity of $5\text{E}4$). The precursor ions were selected in the quadrupole with
951 an isolation window of 0.7 Da and accumulated to an AGC target of $1\text{E}4$ or a maximum
952 injection time of 50 ms and activated using CID fragmentation (35% NCE). The fragments were
953 analyzed in the Ion Trap Analyzer at the turbo scan rate. The 10 most intense MS2 fragments
954 were selected in the ion trap using MS3 multi-notch isolation windows of 2 m/z . An orbitrap
955 resolution of 60k was used with an AGC target of $1.0\text{e}5$ or a maximum injection time of 118
956 ms and activated using HCD fragmentation (65% NCE). QCloud was used to control instrument
957 longitudinal performance during the project.⁽⁶⁰⁾

958 Data analysis

959 LC-MS/MS runs of all 18 samples were searched together using the MaxQuant algorithm
960 (version 2.1.3.0) with mainly default search settings, including a false discovery rate set at 1%
961 at the peptide and protein levels. Spectra were searched against the mouse protein sequences in
962 the Swiss-Prot database (database release version of January 2022), containing 21,986
963 sequences (www.uniprot.org). The mass tolerance for precursor and fragment ions was set to
964 4.5 and 20 ppm, respectively, during the main search. Enzyme specificity was set to the C-
965 terminus of arginine and lysine, also allowing cleavage at Arg/Lys-Pro bonds with a maximum
966 of two missed cleavages. Variable modifications were set to oxidation of methionine residues
967 and acetylation of protein N-termini whereas carbamidomethylation of cysteine residues was
968 set as a fixed modification. Only proteins with at least one unique or razor peptide were retained,
969 leading to the identification of 4,036 proteins. MS3-based quantification using TMTpro labels
970 was chosen as the quantification method, and a minimum ratio count of 2 unique peptides was
971 required for quantification. Further data analysis of the shotgun results was performed with an
972 in-house R script using the proteinGroups output table from MaxQuant. Reverse database hits
973 were removed, reporter intensities were log₂ transformed, and median-normalized and replicate
974 samples were grouped. Proteins with less than three valid values in at least one group were
975 removed and missing values were imputed from a normal distribution centered around the
976 detection limit (DEP package), leading to a list of 4,033 quantified proteins in the experiment,
977 used for further data analysis.⁽⁶¹⁾ To compare protein abundance between pairs of sample
978 groups, statistical testing for differences between two group means was performed using the
979 limma package.⁽⁶²⁾ Statistical significance for differential regulation was set by a raw p<0.05
980 and fold change |log₂FC|≥0.5. The mass spectrometry proteomics data have been deposited to
981 the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier
982 PXD038815.

983

984 **CRISPR/Cas9-mediated deletion of *PLAAT3***

985 The lentiviral plasmid plentiCRISPRv2 was a gift from the Zhang lab (Addgene, MA, USA;
986 plasmid #52961) and contains hSpCas9, a guide RNA (gRNA), and a puromycin resistance
987 sequence. The gRNA targeting exon 2 of *PLAAT3* was designed and checked for efficiency
988 (<http://cistrome.org/SSC>) and specificity (<http://crispr.mit.edu>). The web-based tool CRISPOR
989 (<http://crispor.tefor.net>) was used to evaluate potential off-target sequences. Subsequently, the
990 gRNA was cloned into plentiCRISPRv2, and lentiviruses were produced by the VVTG platform

991 (SFR Necker, France). ASCs were infected with viral particles at a minimal titer of 10^8
992 transducing units per mL. Forty-eight hours postinfection, the cells were selected with 5 $\mu\text{g}/\text{mL}$
993 puromycin dihydrochloride (#P9620; Sigma-Aldrich). Surviving cells were propagated and the
994 heterogeneous cell pool was used for experiments. The percentage of on-target recombination
995 including insertions and deletions (indels) in the genomic DNA from the KO cells was evaluated
996 by Sanger sequencing followed by analysis using the Synthego web-based tool
997 (<https://ice.synthego.com>). The gRNA sequence used in this study is provided in
998 Supplementary Table 7.

999

1000 **Cell culture**

1001 Human ASCs were isolated from surgical samples of subcutaneous abdominal adipose tissue
1002 from a control woman with normal BMI. Adipose tissue samples were enzymatically digested
1003 with collagenase B (0.2%). After centrifugation, the stromal vascular fraction was filtered,
1004 rinsed, plated and cultured in α -MEM with 10% FCS, 2 mmol/L glutamine, 1% P/S (10,000
1005 UI/mL), 1% HEPES and Fibroblast Growth Factor-2 (145 nmol/L). After 24 h, only ASCs
1006 adhered to plastic surfaces, while other cells were removed after culture medium replacement.
1007 ASCs were maintained in an undifferentiated state in high-glucose (4.5 g/L) DMEM
1008 supplemented with 10% newborn calf serum and 1% P/S. All culture conditions were kept
1009 constant throughout the experiments. ASC differentiation was induced as described
1010 previously.^(29, 35) Briefly, 2-day postconfluent cultures were treated with high-glucose (25
1011 mmol/L) DMEM supplemented with 10% FCS, 1% P/S, 1 $\mu\text{mol}/\text{L}$ dexamethasone, 1 $\mu\text{mol}/\text{L}$
1012 rosiglitazone, 250 $\mu\text{mol}/\text{L}$ IBMX and 0.17 $\mu\text{mol}/\text{L}$ insulin for ten days. The medium was then
1013 replaced with high-glucose DMEM supplemented with 10% FCS, 1% P/S, 1 $\mu\text{mol}/\text{L}$
1014 rosiglitazone and 0.17 $\mu\text{mol}/\text{L}$ insulin and changed to fresh medium every 2 days until the 20th
1015 day.

1016

1017 **Oil Red-O staining, image processing, and quantification**

1018 Intracellular lipids were stained with Oil Red-O (#O0625; Sigma-Aldrich). Cells were washed
1019 with PBS and fixed with 4% PFA in PBS for 10 min. Fixed cells were incubated with Oil Red-
1020 O solution for 1 h at room temperature and then with DAPI (#D1306, Thermo Fischer
1021 Scientific) for 5 min. Fluorescence images were generated with an IX83 Olympus microscope,
1022 acquired with Cell-Sens V1.6 and analyzed with FIJI software. Images of 8-10 different areas
1023 per condition were visualized by fluorescence microscopy using mCherry and DAPI filters,
1024 followed by computer image analysis using FIJI software. Briefly, analysis was performed by

1025 threshold converting the 8-bit red-green-blue image into a binary image, which consists only of
1026 pixels representing lipid droplets (*i.e.*, red). Importantly, after separation, the binary image was
1027 manually compared with the original image for consistency and correct binary conversion. The
1028 area occupied by lipid droplets in the image was displayed by FIJI software as surface area in
1029 μm^2 and normalized to cell number by semiautomated counting of DAPI-stained nuclei.

1030

1031 **Quantification of intracellular triglyceride content**

1032 Intracellular lipids were extracted from differentiated ASC using hexane/isopropyl alcohol
1033 (3:2). Cells were washed and incubated with hexane/isopropyl alcohol (3:2, vol/vol) using 500
1034 μL per well in 6-well culture plates on a shaker (80 rpm/minute) at room temperature for 60
1035 minutes. The content of each well was then transferred into a glass tube for nitrogen evaporation
1036 of the organic solvent. After evaporation, the lipids were resuspended in isopropyl alcohol and
1037 transferred into duplicate 96-well plates for analysis after drying. Triglycerides were measured
1038 using the Infinity™ Triglyceride kit (Thermo Fischer Scientific) according to manufacturer's
1039 instructions. The absorbance of each well was measured using a Tecan microplate reader
1040 (TECAN, Männedorf, Switzerland) and converted to concentration based on a standard curve.
1041 The results were normalized to the cell protein content.

1042

1043 **Western blot**

1044 Cells were homogenized in NP-40 lysis buffer to obtain protein lysates. Thirty micrograms of
1045 protein extracts were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis
1046 (SDS-PAGE), transferred to a polyvinylidene difluoride membrane and analyzed by
1047 immunoblotting. Adipose tissues were dissociated and homogenized with ceramic beads and
1048 NP-40 lysis buffer using a Bead Ruptor (#19-042E, OMNI International, GA, USA). Western
1049 blot quantification was performed in triplicate using Fiji software (Open source), and the results
1050 were normalized to the tubulin, ERK or AKT protein levels.

1051

1052 **Data availability**

1053 All relevant data generated and analyzed in this study are included in the article. Mouse RNA-
1054 seq data have been deposited in the Gene Expression Omnibus (GEO) at NCBI (GSE233433).
1055 The mouse mass spectrometry proteomics data have been deposited to the ProteomeXchange
1056 Consortium via the PRIDE partner repository with the dataset identifier PXD038815. The
1057 human and mouse lipidomics data are available as supplementary files (Plaat3 mouse

1058 lipidomics.html; Plaatz mouse mediator lipidomics.html; PLAAT3 human lipidomics.html).

1059 For reasons of privacy, clinical patient sequencing data are not publicly available..

1060

1061 **Code availability**

1062 All multi-omics related software used in this study is published and cited either in the main
1063 text or Methods. No custom code was used for data processing or analysis of the
1064 transcriptomics, proteomics or lipidomics datasets. Data analysis approaches using published
1065 software packages are described in the Methods and Supplementary Notes. For patients'
1066 privacy reasons the Seqplorer codes for variant calling and filtering won't be publicly available.

1067

1068 **Methods-only references**

- 1069 44. Slifer SH. PLINK: Key Functions for Data Analysis. *Curr Protoc Hum Genet.*
1070 2018;97(1):e59 <https://doi.org/10.1002/cphg.59>.
- 1071 45. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and
1072 guidelines for the interpretation of sequence variants: a joint consensus recommendation of the
1073 American College of Medical Genetics and Genomics and the Association for Molecular
1074 Pathology. *Genet Med.* 2015;17(5):405-24 <https://doi.org/10.1038/gim.2015.30>.
- 1075 46. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence
1076 Alignment/Map format and SAMtools. *Bioinformatics.* 2009;25(16):2078-9
1077 <https://doi.org/10.1093/bioinformatics/btp352>.
- 1078 47. Rausch T, Zichner T, Schlattl A, Stütz AM, Benes V, Korbel JO. DELLY: structural
1079 variant discovery by integrated paired-end and split-read analysis. *Bioinformatics.*
1080 2012;28(18):i333-i9 <https://doi.org/10.1093/bioinformatics/bts378>.
- 1081 48. Layer RM, Chiang C, Quinlan AR, Hall IM. LUMPY: a probabilistic framework for
1082 structural variant discovery. *Genome Biol.* 2014;15(6):R84 [https://doi.org/10.1186/gb-2014-](https://doi.org/10.1186/gb-2014-15-6-r84)
1083 [15-6-r84](https://doi.org/10.1186/gb-2014-15-6-r84).
- 1084 49. Chen X, Schulz-Trieglaff O, Shaw R, Barnes B, Schlesinger F, Källberg M, et al. Manta:
1085 rapid detection of structural variants and indels for germline and cancer sequencing
1086 applications. *Bioinformatics.* 2016;32(8):1220-2
1087 <https://doi.org/10.1093/bioinformatics/btv710>.
- 1088 50. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler
1089 transform. *Bioinformatics.* 2009;25(14):1754-60
1090 <https://doi.org/10.1093/bioinformatics/btp324>.
- 1091 51. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A
1092 framework for variation discovery and genotyping using next-generation DNA sequencing data.
1093 *Nat Genet.* 2011;43(5):491-8 <https://doi.org/10.1038/ng.806>.
- 1094 52. Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, et al. A program for
1095 annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the
1096 genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly (Austin).* 2012;6(2):80-92
1097 <https://doi.org/10.4161/fly.19695>.
- 1098 53. Cingolani P, Patel VM, Coon M, Nguyen T, Land SJ, Ruden DM, et al. Using
1099 *Drosophila melanogaster* as a Model for Genotoxic Chemical Mutational Studies with a New
1100 Program, SnpSift. *Front Genet.* 2012;3:35 <https://doi.org/10.3389/fgene.2012.00035>.

1101 54. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and
1102 genotyping with HISAT2 and HISAT-genotype. *Nat Biotechnol.* 2019;37(8):907-15
1103 <https://doi.org/10.1038/s41587-019-0201-4>.

1104 55. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. StringTie
1105 enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol.*
1106 2015;33(3):290-5 <https://doi.org/10.1038/nbt.3122>.

1107 56. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for
1108 differential expression analysis of digital gene expression data. *Bioinformatics.*
1109 2010;26(1):139-40 <https://doi.org/10.1093/bioinformatics/btp616>.

1110 57. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. KEGG for integration and
1111 interpretation of large-scale molecular data sets. *Nucleic Acids Res.* 2012;40(Database
1112 issue):D109-14 <https://doi.org/10.1093/nar/gkr988>.

1113 58. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al.
1114 Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat*
1115 *Commun.* 2019;10(1):1523 <https://doi.org/10.1038/s41467-019-09234-6>.

1116 59. Maia TM, Staes A, Plasman K, Pauwels J, Boucher K, Argentini A, et al. Simple Peptide
1117 Quantification Approach for MS-Based Proteomics Quality Control. *ACS Omega.*
1118 2020;5(12):6754-62 <https://doi.org/10.1021/acsomega.0c00080>.

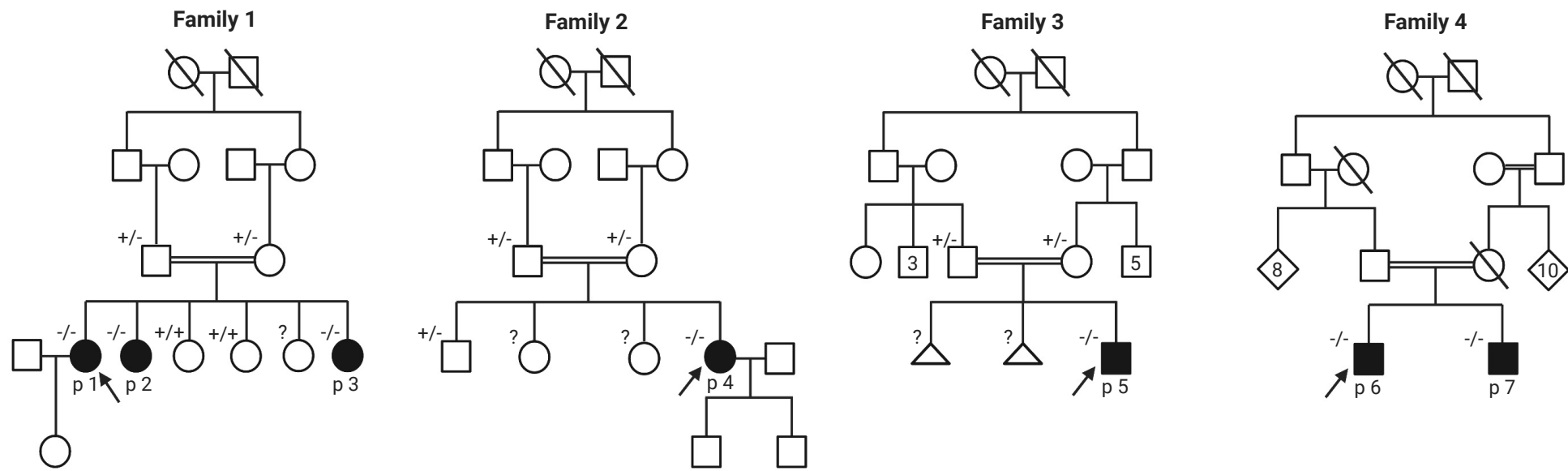
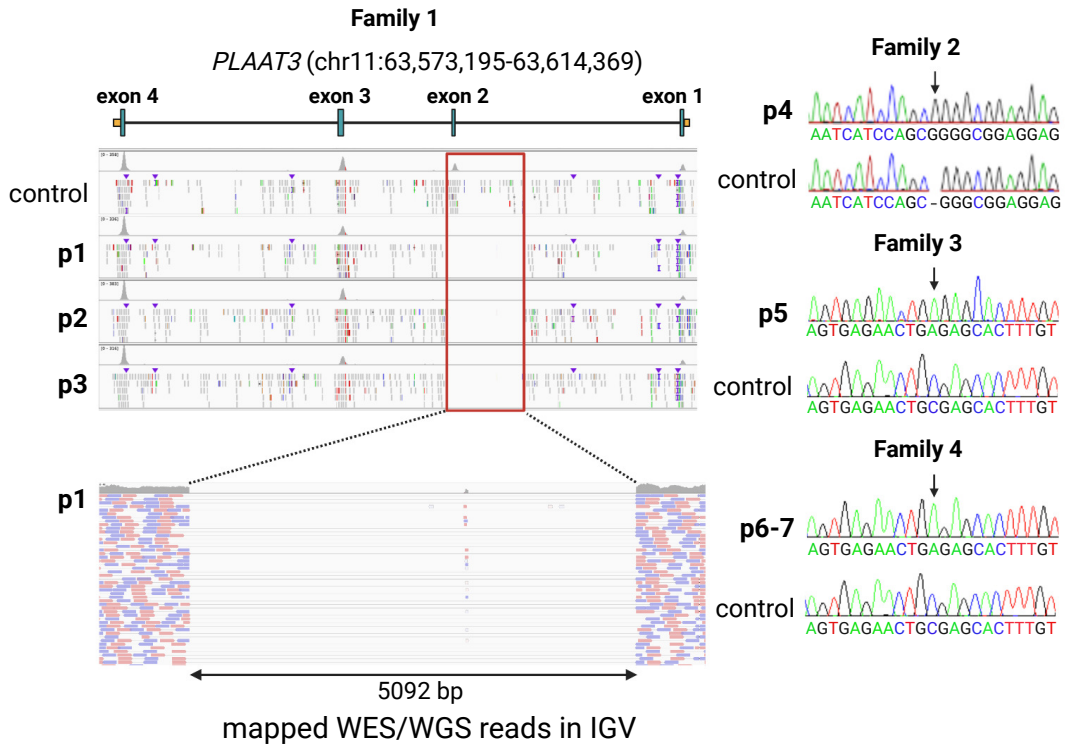
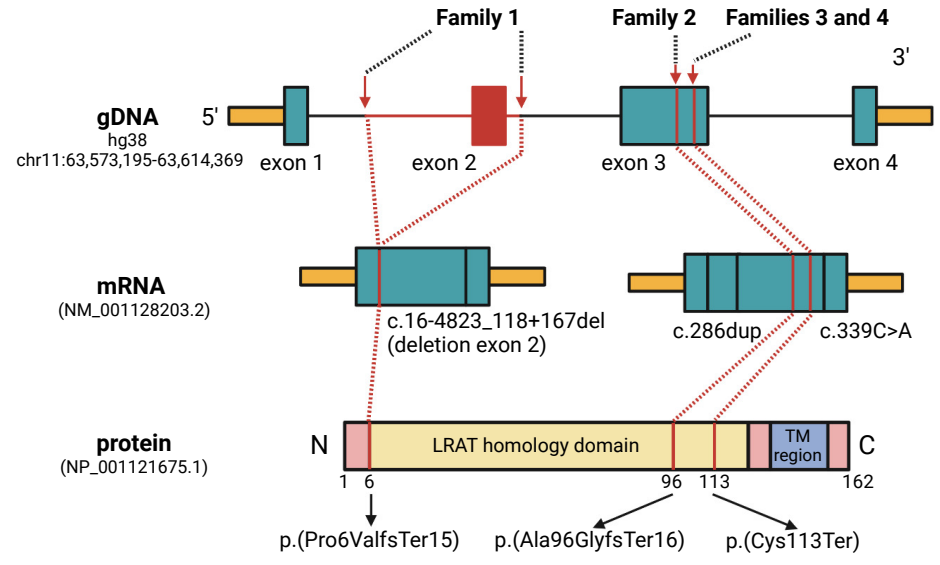
1119 60. Chiva C, Olivella R, Borràs E, Espadas G, Pastor O, Solé A, et al. QCloud: A cloud-
1120 based quality control system for mass spectrometry-based proteomics laboratories. *PLoS One.*
1121 2018;13(1):e0189209 <https://doi.org/10.1371/journal.pone.0189209>.

1122 61. Zhang X, Smits AH, van Tilburg GB, Ovaa H, Huber W, Vermeulen M. Proteome-wide
1123 identification of ubiquitin interactions using UbIA-MS. *Nat Protoc.* 2018;13(3):530-50
1124 <https://doi.org/10.1038/nprot.2017.147>.

1125 62. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential
1126 expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.*
1127 2015;43(7):e47 <https://doi.org/10.1093/nar/gkv007>.

1128

1129

a**b****c**

a

Fat percentage
Low High
25% 60%

